Review

Immunology and immunotherapy of the infections caused by *Pythium insidiosum*

LEONEL MENDOZA* & JOSEPH C. NEWTON†

*Medical Technology Program, Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan, and †Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, Alabama, USA

Although infections caused by the straminipilan pathogen *Pythium insidiosum* were described in 19th century, it has been only recently that its epidemiology, immunology, treatment and other important traits were extensively studied. These studies were of paramount importance to theorize about the ecological niche for this pathogen, its host-parasite relationships, the antigens used for diagnosis, and the management of the infection using immunotherapy. *P. insidiosum* triggers in the infected host a T helper 2 [Th2] subset with an inflammatory reaction composed mainly of eosinophils and mast cells. These cells degranulate around the hyphal elements of *P. insidiosum* where a Splendore-Hoeppli-like reaction develops. In horses this reaction is so intensive that firm concretions called ‘kunkers’ develop. These data indicated that this pathogen might have developed an evolutionary strategy to conceal important antigens from the host immune system. Immunotherapy, a treatment approach that relies on the injection of antigens of *P. insidiosum* from *in vitro* cultures, has been successfully used in humans and horses to manage this disease. A switch from a Th2 to Th1 response is postulated as the most likely explanation of the curative properties of this approach. This review provides details on the serological, immunological, and immunotherapeutic methodologies used to diagnose and treat the infections caused by this pathogen.

**Keywords** *Pythium insidiosum*, pythiosis, immunotherapy

---

History

*Pythium insidiosum* is the cause of the disease pythiosis, a chronic, pyogranulomatous disease of horses, dogs, cattle, cats and humans [1]. The organism belongs to the Kingdom Straminipila, Phylum Oomycota, Family Pythiaceae, Genus *Pythium* and species *insidiosum* [2]. There are at least 120 known *Pythium* species distributed throughout the world. Most *Pythium* species are soil inhabitants and some are important plant pathogens [3]. Pythiosis in animals occurs in tropical, subtropical and temperate areas and has been reported in Argentina, Australia, Brazil, Colombia, Costa Rica, Egypt, Greece, Haiti, India, Indonesia, Japan, Papua New Guinea, Thailand and the US [4].

The first reports of what is believed to be pythiosis were published in the mid to late 1800s associated with lesions in horses. These early diagnoses are controversial, however, because of the similarity of the gross lesions of pythiosis and equine cutaneous habronemiasis [5], a malady of horses caused by larvae of spirurid gastric parasites of the Genus *Habronema* [5]. Additionally, the two conditions shared common names in various countries during this time period (mid to late 1800s to early 1900s); e.g., both were called swamp cancer in Australia, bursatti in India, leeches in the US...
and granular dermatitis in Japan [4]. It is believed that the first published descriptions of pythiosis were those of Smith [6] and Drouin [7] in the late 1800s. In both of these reports the authors commented on the presence of mycelial elements in the lesions [1]. The first definitive evidence of *Pythium* being caused by a ‘fungal’ organism was presented in 1895 by Fish [8] when he described hyphae that had the characteristic histologic appearance of an unnamed phycymycete in tissue sections from Florida horses with ‘leeches’. Also in the late 1800s, Theodore Smith [9] described a disease known locally as ‘leeches’ because the firm elongated masses within cutaneous/subcutaneous lesions on the lower legs were thought to be aquatic leeches that had invaded the tissues as the animal stood in water. In 1901, de Haan and Hoogkaner [10] published extensive descriptions of several cases of what is believed to be pythiosis in Indonesian horses and named the disease hyphomycosis destructuens. They also reported the isolation of a ‘fungal organism’ from these lesions. In a subsequent publication in 1902, de Haan [11] renamed the disease hyphomycosis destructuens equi. The organism was isolated a second time by Witkamp in 1924 [12]. In 1961, the organism was given the name *Hyphomyces destructuens* by Bridges and Emmons [13]. They considered the organism to be a phycymycete, possibly in the Genus *Mortierella*, based on its tissue morphology, as well as its broad, branched, sparsely septate, nonsporulating mycelium in culture. The authors did not follow the established rules of naming a new organism as outlined in the International Code of Botanical Nomenclature and the name *H. destructuens* was not accepted [1]. The conclusion of Bridges and Emmons that *H. destructuens* should be considered a phycymycete, was reinforced by Japanese workers using isolates from horses with granular dermatitis [14,15].

In 1974, Austwick and Copland [16] observed that isolates of *H. destructuens* isolated from horses in New Guinea produced biflagellated motile zoospores. They suggested that the organism was a phycymycete belonging to the Pythiaceae and that it could be included in the genus *Pythium*. In 1980, Ichitani and Amemiya [17] isolated an organism from a horse in Japan and suggested that the organism was *Pythium gracile*. Two publications in 1987 also placed the organism in the genus *Pythium*. In 1987, DeCock et al. [1] working with isolates from animals (including the Japanese isolate above) found the organism to be the same in all and proposed the binomial *Pythium insidiosum*. Shipton [18], working with Australian equine isolates and also the Japanese isolate above, proposed the binomial *Pythium destructuens*. Most publications concerning this disease since 1987 have referred to the organism as *P. insidiosum*.

**Pythiosis in animals**

The disease has been described in a variety of animals in countries throughout the world. Most published accounts of the disease have been from horses and dogs. In horses, the disease manifests as an ulcerative, proliferative, pyogranulomatous lesion most often involving skin and subcutaneous tissue of the legs and ventral body wall [19] (Fig. 1a). The disease has also been reported in the equine lung [20], bone [21] and intestine [22]. Systemic involvement in three horses has been described [23]. The organism causes infection in dogs, which include cutaneous/subcutaneous and gastrointestinal lesions [24,25]. The gastrointestinal presentation is most common in the dog [26,27]. Other reported cases have been described from domestic cats (nasal cavity/retrobulbar space, skin/subcutaneous tissues) [27–29], cattle (skin/subcutaneous) [30], sheep (skin/subcutaneous, disseminated) [31], a dromedary camel (skin, gastric) [32] and a Central American Jaguar (lung) [33].

**Pythiosis in humans**

In 1985, the first cases of human pythiosis were reported from patients living in Thailand [34]. Other human cases were described over the next 15 years [35–43]. Three forms of human pythiosis have been observed: (i) granulomatous and ulcerative lesions involving the skin and subcutaneous tissues of the limbs and face, including the periocular area, (ii) Ophthalmic pythiosis causing keratitis, and (iii) systemic pythiosis with vascular involvement leading to vasculitis, thrombosis and aneurysms [37,38,42]. Risk factors that appear to be involved in human pythiosis include hemoglobinopathy (thalassemia) and occupations requiring work in aquatic habitats (rice-fields, etc.) [41].

**Evaluating the immune response in mammalian hosts with pythiosis**

The first serological assays to diagnose pythiosis in equines were developed early in the 20th century [12]. They were immunodiffusion (ID, precipitin), complement fixation (CF) and a skin test. It was clearly demonstrated that horses with active infection developed a cell mediated hypersensitivity reaction and the production of specific antibodies against the purified antigens of *P. insidiosum*. However, due to the association of pythiosis with other skin diseases of horses at
fluorescent-antibody assay that differentiated the hyphae of *P. insidiosum*.

Their serological tools accurately diagnosed equine pythiosis. In 1924 [12], these authors indicated that different antigens based on the data published by Witkamp [12]. These authors indicated that their immunological tool was initially developed to analyse the antigens of *P. insidiosum* in tissue. Although they did not adsorb their reagent, no cross-reaction was detected with this assay when tested with the hyphae of three different zygomycetes. These two assays have been contributions in the identification of the hyphae of *P. insidiosum* in tissue, especially when culture of the organism is not possible. Another test, immunoblot, was introduced in 1992 [48]. This immunological tool was initially developed to analyse the antigens of *P. insidiosum* detected by the IgG-humoral immune response during infection. However, this strategy has also been recently used as a specific diagnostic tool to diagnose pythiosis in different species [55–58]. Owing to the insensitivity of the ID test and cross-reactivity of the CF and skin test, an ELISA test was developed [55,56]. Investigators have found ELISA to be specific and sensitive in detecting pythiosis in all infected hosts [43,57–59]. These serological tests were of paramount importance in the characterization of the humoral response to *P. insidiosum* during infection, and as a diagnostic tool for the disease. These findings suggest that the anti-*P. insidiosum* humoral response is also stimulated in infected hosts with pythiosis. The finding, using an ELISA test, that serum IgE changed before and after *Pythium* immunotherapy is one example on how this test has increased our knowledge of the humoral response to *P. insidiosum* antigens in horses. More recently, Hutchens and Mendoza [60] introduced a simple latex agglutination test for the rapid diagnosis of pythiosis. This new test was more sensitive than the ID test, but specificity was low. The data so far collected from serological studies have been instrumental in explaining the cascade of immunological events triggered by the antigens of *P. insidiosum* in infected and vaccinated hosts (see below) [46].

**Pythium insidiosum** host–parasite relationship

*Pythium insidiosum* cannot penetrate healthy skin. The studies of Ravishankar *et al.* [61] showed that the hyphal apices of this pathogen exerted forces of up to 6.9 μN when tested against different surfaces, including human and animal skin. However, this force is not strong enough to penetrate healthy skin. The study showed that this pathogen requires prior skin damage to invade its mammalian host. Once inside the host,
*P. insidiosum* triggers an eosinophilic granulomatous reaction with giant cells, mast cells, macrophages, plasma cells, and a few lymphocytes [4,19]. This reaction is similar to that observed in infections caused by the fungal pathogens Basidiobolus ranarum and Conidiobolus coronatus [5] and also to the reactions seen in many parasitic infections [5]. Because these two entomophthoraceous fungi and some parasitic infections mimic the clinical manifestations of the infections caused by *P. insidiosum* in both humans and animals, an accurate differentiation between these pathogens is essential for the proper diagnosis and treatment [4,5]. Since the first report of pythiosis in horses it was evident that *P. insidiosum* causes an eosinophilic reaction leading to the development of a Splendore-Hoeppli like material around hyphae in tissues. In horses the reaction is so pronounced that the eosinophils heavily degranulate around the hyphae of *P. insidiosum* forming the firm concretions termed ‘kunkers’ [4,19,62]. ‘Kunkers’ have not been found in other species affected by this oomycetous pathogen [27,28,30,31,34,35,38]. The infection in humans and animals indicated that *P. insidiosum* triggers a prominent eosinophilic inflammatory reaction, as a response to antigens released by the hyphae invading the infected hosts [4,27,38]. It is interesting to note that the hyphal elements of *P. insidiosum* are usually found in the center of eosinophilic micro-abscesses. This feature has been used by some investigators to postulate that a Th2 subset is probably activated by the immunogens expressed by *P. insidiosum* in the infected hosts [46].

The pathogenesis of the disease has been linked, in part, to the degranulation of eosinophils and mast cells around the hyphae of *P. insidiosum*, although other immunological mediators have also been incriminated [44,46,47]. It is believed that the degranulation of these inflammatory cells is largely responsible for the extensive tissue damage observed in humans and animals with pythiosis. Clinically, extensive ulceration of the skin accompanied by the production of large amounts of granulation and fibrous tissue are main characteristic of the cutaneous infections [4,5,19,44]. In horses the lesions are so extensive that the disease has been termed ‘swamp cancer’ or ‘summers sores’. Skin lesions in horses can reach 500 cm in diameter [63]. When *P. insidiosum* invades internal organs the development of tumor-like masses, often misdiagnosed as neoplasms, is the main feature [4,5,27,28,30]. Further evidence that a Th2 response has been triggered by *P. insidiosum* immunogens, and thus presented to local dendritic cells, came from the studies of Wachiwanawin *et al.* [41]. These authors reported that in humans with arterial pythiosis, interleukin-4 (IL-4) and IL-5 were elevated. The subsequent finding of elevated IgE levels in humans and horses with the disease strongly validated the concept of a Th2 modulation by this pathogen during natural infection.

Under this scenario, the hyphae of *P. insidiosum* express a soluble exoantigen that modulates the host’s immune response to a Th2 subset. The expression of such antigen released outside the cytoplasmic compartment, was first recorded in 1987 [53]. These investigators reported that when some ‘kunkers’, embedded in infected equine tissues, were investigated with fluorescent antibodies, the whole ‘kunker’ fluoresced. This finding suggested that an antigen, detected by the labeled anti-*P. insidiosum* immunoglobulin, had been released from the hyphae. Apparently, some of the antigen is retained in the ‘kunkers’, from where it is released to the surrounding tissues and then presented to local dendritic cells. It has been suggested that this soluble antigen may be responsible, not only for the immune modulations to a Th2 subset, but it also might play a key role on locking the immune system into a Th2 subset. This hypothesis also suggests that the exoantigens released by *P. insidiosum* may play an important role in the development of pathological changes observed during pythiosis [46]. We believe that the host’s immune system gets deceived by *P. insidiosum* exoantigens. Because the host immune system is always detecting the antigens that this pathogen presents to the local dendritic cells, the host is always producing more eosinophils and mast cells. Furthermore, the pathogen uses the host’s defenses to camouflage itself inside the eosinophilic material formed by the eosinophil degranulation. This evolutionary strategy could protect the pathogen from being fully presented to the host’s immune system [45–47], thus reassuring its presence in the infected tissues. Based on the destructive effect of the inflammatory response triggered by this pathogen on the infected hosts, and the fact that it is the only mammalian oomycete pathogen, it could be speculated that *P. insidiosum* only recently developed the ability to survive in mammalian tissues, a belief also supported by current phylogenetic analysis [64].

**An animal model to evaluate the immune response triggered by Pythium insidiosum and its antigenic therapeutic features**

It is well known that the species frequently diagnosed with pythiosis are resistant to experimental infection by *P. insidiosum* [4]. In fact, the only susceptible animal model for experimental pythiosis, is the rabbit [12,65,66]. This is very unusual since natural pythiosis
in rabbits has yet to be reported. The first investigator that successfully reproduced pythiosis in rabbits was Witkamp [12]. He induced eosinophilic granulomas in the rabbits after intraperitoneal or intravenously injections of the \textit{P. insidiosum} hyphae isolated from horses with cutaneous pythiosis found in India. He reported the same results after placing ‘kunkers’, recovered from the infected horses, in the rabbit’s tissues. He reported that the sera from experimentally infected rabbits reacted positively when tested in a CF test. Amemiya in 1969 and 1982 [15,65] reported the successful reproduction of pythiosis in rabbits using hyphae of \textit{P. insidiosum} isolated from Japanese horses. This investigator stated that he was able to reproduce cutaneous, intestinal, arterial, and systemic pythiosis in experimental rabbits. In the 1982 report, he illustrated his finding with beautiful color plates showing microscopic and macroscopic details of his experiments. One year later Miller and Campbell [66] were the first investigators to incriminate \textit{in vitro} developed zoospores, as the putative infecting units of this oomycete pathogen. These investigators used zoospores from \textit{P. insidiosum} to reproduce the infection in healthy and cortisone-treated rabbits. Their data showed that both groups of rabbits were equally infected by these zoospores, suggesting that \textit{P. insidiosum} could cause infection even in immunocompetent rabbits. Patino-Meza [67] in an effort to reproduce pythiosis in susceptible species using zoospores was unsuccessful in causing disease when zoospores were injected into horses and dogs. However, he was able to induce pythiosis when injected into rabbits. Data collected in these studies suggested that the zoospores, and other propagules of \textit{P. insidiosum}, might require unknown elements to initiate pythiosis in the contacted mammalian hosts.

Since then other investigators have used rabbits to study the cell mediated response induced by \textit{P. insidiosum} and for the production of immunoglobulins to evaluate and develop diagnostic tools [49,50,54,59,68,69]. Santurio \textit{et al} [59] used rabbits to evaluate three different formulations used for immunotherapy. After first inducing pythiosis he injected the sick rabbits with three immunotherapeutic antigens derived from \textit{P. insidiosum}. Using this strategy, they not only evaluated these three immunogens, but their results suggested that the response to immunotherapy could be easily monitored using the rabbit model. More recently, rabbits have been used to evaluate the immune-prophylactic potential of a variety of \textit{P. insidiosum} immunogens (B. Glass, personal communication).

The use of immunotherapy to treat pythiosis in humans and animals

\textit{Pythium insidiosum} is resistant to most antifungal drugs [4]. One explanation for this could be the fact that this oomycete lacks ergosterol in its cytoplasmic membrane. Because of this, surgery and other less traditional drugs and methods have been used for the treatment of this disease, often with less than satisfactory results [4,5,27,36,38]. One of the new treatments has been the use of \textit{P. insidiosum}-derived antigens in immunotherapy against pythiosis.

As previously mentioned, Witkamp [12] first used a skin test to evaluate the cellular immunity in horses. However, he did not mention if a cure was achieved after this experiment. Miller [46] was the first investigator to report that the culture-derived antigens of \textit{P. insidiosum} possessed therapeutic properties when injected into horses. The same year another group described a similar finding in Costa Rican horses [63]. While using culture-derived \textit{Pythium} immunogens in skin tests to investigate the epidemiology of equine pythiosis these two groups independently found that the injected immunogens of this oomycete pathogen resulted in the cure of \textasciitilde{}50\% of the treated animals. Miller \textit{et al} [70] in a later report questioned the effectiveness of this new immunological treatment (immunotherapy) when some of the horses injected failed to improve. Other investigators reported that \textit{P. insidiosum} immunogens were not 100\% effective in chronic cases [47]. Later work using the same antigens as Miller [45], and those used by Mendoza \textit{et al} [63], showed that 100\% of early cases of the disease (<20 day old lesions) were cured, whereas only 20–40\% of the chronic cases responded to immunotherapy (>2 months old lesions). Since then, immunotherapy has been successfully used in more than 500 hundred equine cases of pythiosis [46]. Using a new immunotherapeutic formulation, Mendoza \textit{et al} [46] reported 60\% of effectiveness in horses subjected to immunotherapy alone (Fig. 1, Table 1). The combination of surgical debridement of diseased tissue followed by immunotherapy increased the cure rate to approximately 90\% when tested on several cases of chronic cutaneous pythiosis in horses. In addition, immunotherapy has recently been successful in cattle with pythiosis. However, no published data are available. In cattle, the effectiveness of immunotherapy on disease cure rate approached 100\% (R. C. Pérez, personal communication).

In contrast to the results obtained in horses and cattle, immunotherapy in cats and dogs has been disappointing. Only 33\% of the treated dogs and...
none of the five cats treated to date responded successfully to immunotherapy [27,46,71] (Table 1). The cause of this failure is unknown but one explanation might be that most dogs and cats with pythiosis are diagnosed several months after the initial onset of infection resulting in animals with weakened immune systems that poorly respond to immunotherapy. The two classical clinical forms (skin and gastrointestinal) of the disease in dogs mimic several cutaneous and intestinal diseases [25,27–29], thus complicating the differential diagnosis. The failure of an accurate clinical diagnosis and the fact that most clinicians are unfamiliar with the disease, may explain why most cases are initially misdiagnosed as cutaneous bacterial or fungal infections or as intestinal neoplasms such as lymphoma [27].

In addition to horses, dogs and cattle immunotherapy has been successfully tested in human patients in Thailand suffering with arterial pythiosis [39,41]. This type of pythiosis in humans has had a 100% mortality rate. In the first case of immunotherapy for pythiosis, a young boy with terminal pythiosis in his carotid artery was successfully treated with *Pythium* immunotherapy [39]. He had failed to respond to antifungal therapy and was treated with immunotherapy on an experimental basis. The dramatic recovery of this boy after immunotherapy was surprising. The boy reacted with a mild inflammatory response at the site of injection that subsided two days post treatment. No deleterious side effects were reported after two injections with *P. insidiosum* immunogens. This first human case cured by immunotherapy suggested that this treatment approach might be an effective and safe method of treatment of human arterial pythiosis. Recently, Wachianawanin *et al.* [41] using a new formulation described previously in horses, dogs, and a human, found that this approach successfully cured four of eight Thai cases of terminal arterial pythiosis (Table 1). This study indicates that immunotherapy should be considered in cases of cutaneous and arterial pythiosis in humans. Although immunotherapy has yet to be tested in the cutaneous form, these data indicate that it is a safe approach for any clinical form of pythiosis in humans.

Data collected in the US using *P. insidiosum*-immunotherapy as a curative option for animals, was approved by USDA as an experimental option for diseases with or without chemotherapeutic substitutes. In Thailand most physicians used this curative approach as a last resort in cases of human arterial pythiosis. As mentioned earlier, only rabbits are susceptible to experimental pythiosis. Thus, the majority of information on immunotherapy has been collected from naturally infected treated hosts. Although, this could initially appear as a disadvantage, it could be more powerful than data collected in an animal model. Accordingly, data collected from clinical trials in the past 10 years indicated that: (i) that immunotherapy is safe, in both human and animals, (ii) it cures ~60% of treated hosts, and (iii) it appears to modulate the host immune response in treated recipients. Data collected in the last two decades suggest that this new concept

---

**Table 1** Efficacy of immunotherapy in humans and animals with clinical pythiosis

<table>
<thead>
<tr>
<th>Immunotherapy*</th>
<th>Cattle§</th>
<th>Dogs¶</th>
<th>Horses*</th>
<th>Humans†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cured</td>
<td>97.0%</td>
<td>33.1%</td>
<td>60.0%</td>
<td>55.5%</td>
</tr>
<tr>
<td>Total number treated</td>
<td>37</td>
<td>21</td>
<td>~600</td>
<td>9</td>
</tr>
</tbody>
</table>

*The immunotherapeutic agent used in each of the injected hosts was a mixture of high molecular weight exo- and endoplasmic antigens of *Pythium insidiosum* [41,46].

§, Rosa Cristina Pérez, personal communication; ¶, Unpublished data; *, Unpublished data; †, [39,41].

---

**Fig. 1** The response of a horse with pythiosis to immunotherapy. Panel A shows an equine hoof before immunotherapy. Panels B and C show the response leading to cure after 5 months of immunotherapy.
could be extrapolated to chronic subcutaneous mycoses and, in principle, to deep mycoses as well.

**Hypothesis behind the curative properties of *Pythium insidiosum* antigens used for immunotherapy**

Immunotherapeutic approaches to infectious disease treatment using microbe-derived antigens have been previously investigated [72–74]. However, the immunogens derived from *P. insidiosum* have been more successful than any of the immunotherapeutic antigens investigated to date in other chronic diseases [39,41,47,59,70–76]. Early reports indicated that a mixture of *in vitro* derived *P. insidiosum* immunogens triggered an immune response that seemed to kill *P. insidiosum* [41,46]. These reports suggested that the injected immunogens displayed to the host’s immune system, antigens that were not properly presented during natural infection to stimulate a protective response. They stated that the development of a mononuclear response and the disappearance of the eosinophilic reaction around the hyphae, after successful immunotherapy, were findings consistent with this idea. Similar observations have been published by Santurio et al. [59] and Monteiro [77] using immunotherapy in horses with pythiosis in Brazil.

Thitithayanont et al. [39], after successfully curing a young boy from Thailand with arterial pythiosis using culture-derived antigens of *P. insidiosum*, speculated that a switch from a Th2 to a Th1 response was the most likely trigger behind the curative properties of this immunotherapeutic approach. This hypothesis was based on the fact that the Th2 subset produces IL-5 and IL-4, as well as, IgE, IgG, and IgM precipitating immunoglobulins, all factors involved in the initiation of hypersensitivity and eosinophilia. In contrast, the Th1 subset produces IL-2 and IFN-γ and it is involved in cytotoxic T lymphocytes (CTL) expansion, macrophage activation, and IgG isotypes mediating complement activation on sensitized pathogens. These authors, and more recently others [46,71,78,79], have indicated that the disappearance of the eosinophilic response and the expression of a mononuclear reaction after immunotherapy of pythiosis

**Fig. 2** The working hypothesis on the immunotherapeutic mechanisms triggered by *Pythium insidiosum* antigens. The left side of the chart shows the events during natural infection. It is believed that exoantigens of *P. insidiosum* (large circles around the hyphae) are processed by antigen presenting cells (APC) and then fully present them to naïve T helper cells (Th0). The Th0 cells differentiate into a T helper 2 (Th2) subset expressing large quantities of IL-5, IL-4, and other Th2 related cytokines. The IL-4 may stimulate B cells to produce IgE and also precipitin IgG and IgM (detected in most serological assays). In turn, IL-5 and IgE stimulate mast cells and eosinophils to migrate to the infection site. These inflammatory cells degranulate around the hyphae (Splendore-Hoeppli phenomenon) causing tissue damage, and in the process masking the hyphae of *P. insidiosum* from the host’s immune system. IL-4 could also down-regulate the Th1 response further locking the host’s response into a Th2 subset (round dotted arrow). After immunotherapy (left side of the chart), the *in vitro* derived antigens of *P. insidiosum* are processed by local APC in a different fashion than during natural infection. After presentation, the Th0 cells developed into a Th1 subset expressing large quantities of interferon gamma (IFN-γ) and IL-2 that trigger a mononuclear cell-mediated immune (CMI) response including putative cytotoxic lymphocytes (CTL) and macrophages. It is believed that the cellular immunity is directly responsible for the killing of *P. insidiosum* hyphae. B cells are also stimulated to produce putative IgG for long-term protection. The continuous production of IFN-γ down-regulates the Th2 response breaking the cycle created by the exoantigens during natural infection (rectangular dotted arrow). Adapted from Mendoza et al. [46].

© 2005 ISHAM, Medical Mycology 43, 477–486
successful immune therapy, strongly support the changes in immune response stated above. More recently, Wananachiwawin et al. [41], suggested that the in vitro derived immunogens of *P. insidiosum* might be presented to the local dendritic cells in a different fashion than during natural infection, thus stimulating a Th1 rather than Th2 response. They also noted higher titers of IL-4 and IL-5, and IgE previous to immunotherapy in a human with arterial pythiosis, and a decrease of these cytokines together with an increase of IL-2, after successful immunotherapy. This finding and those published by others [39,45–47,59,71,79] strongly suggests that a down regulation of a Th2 subset coupled with an up regulation of a Th1 response had occurred. Mendoza et al. [46] also reported low titer of IgE in cured horses after immunotherapy. These investigators found, on histo-pathological studies of the infected tissue before and after immunotherapy, that upon injection, a mononuclear reaction is triggered and that the hyphal elements of *P. insidiosum* are apparently destroyed by these mononuclear inflammatory cells. The damaged hyphal elements were observed as empty tubular structures, only identified as *P. insidiosum* hyphae by specific immunofluorescence staining [46].

Based on these developments [39,41,46,47,77,78], Mendoza et al. [46] recently proposed a hypothesis to explain the mechanisms behind the immunotherapeutic properties of these antigens. A summary of this proposal is shown in Fig. 2. This hypothesis predicts that the development of a CMI response and the down regulation of the Th2 subset might be responsible for the curative properties of the immunogens used for immunotherapy.

**Future directions**

Although most of the immunological studies have been conducted to evaluate crude extracts of *P. insidiosum* in serological assays, the identity of these antigens remains to be determined. For instance, numerous investigators have reported several immunodominant antigens since 1992 [48,50–52,59]. However, studies to investigate their identity and function at the molecular level has not yet been possible. Their identification is a logical next step. It is likely that the cloning and sequencing of these important antigens will take place in the near future, which will allow for better characterization of the immunogens. The use of these immunogens in the rabbit model should lead to a more detailed and complete picture of the immunopathology and lesion genesis of pythiosis and also give us a better understanding of the immunology involved in successful treatment (or treatment failure) following immunotherapy.

A new understanding of *P. insidiosum* and the disease that it causes should be possible over the next 20 years using modern techniques of immunology and molecular biology. Additionally, a developing awareness in veterinary and human medicine for pythiosis and the development of better diagnostic tests for pythiosis should make early diagnosis of the disease a much more common event. Early diagnosis of the disease and the development and use of improved immunotherapeutic preparations for pythiosis should lead to a better prognosis to this devastating disease of animals and humans.

**References**

8 Fish PA. A historical investigation of two cases of an equine mycosis, with a historical account of the supposed similar disease, called bursatelle, occurring in India. 12th and 13th Annu Rep US Bur Anim Ind 1895–1896; 229–259.
11 de Hann J. Bosartige schemelkrankheit des Pferdes (Hyphomycosis destruens equi). *Zentralbl Bakter Parasitenkld Infektionskr Hyg Abt 1 Orig* 1902; 31: 758–763.
Immunotherapy of pythiosis

56 Rosa PS. Development and evaluation of serological tests to detect pythiosis in horses. MS thesis, Louisiana State University, Baton Rouge, LA, USA. 1993; 1–120.
60 Hutchins M, Mendoza L. Development of a simplified latex agglutination test for the rapid diagnosis of the infections caused by Pythium insidiosum. 102nd General Meeting of the American